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APPLICATION NUMBER: ***09/256,194***
FILING DATE: ***February 24, 1999***

PRIORITY DOCUMENT

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Docket No.
1038-920 MIS:jb

TO THE ASSISTANT COMMISSIONER FOR PATENTS

jc135 U.S. PTO 09/256194 02/24/99

Transmitted herewith for filing under 35 U.S.C. 111 and 37 C.F.R. 1.53 is the patent application of:

Charles D.Y. Sia, et al.

For: EXPRESSING GP140 FRAGMENT OF PRIMARY HIV-1 ISOLATE

Enclosed are:

- ☐ Certificate of Mailing with Express Mail Mailing Label No.
- ☒ Four (4) sheets of drawings.
- ☐ A certified copy of a application.
- ☒ Declaration ☐ Signed. ☒ Unsigned.
- ☐ Power of Attorney
- ☐ Information Disclosure Statement
- ☐ Preliminary Amendment
- ☐ Other:

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra		Rate	Fee
Total Claims	14	- 20 =	0	x	$18.00	$0.00
Indep. Claims	4	- 3 =	1	x	$78.00	$78.00
Multiple Dependent Claims (check if applicable) ☐						$0.00
					BASIC FEE	$760.00
					TOTAL FILING FEE	$838.00

- ☒ A check in the amount of **$838.00** to cover the filing fee is enclosed.
- ☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. **19-2253** as described below. A duplicate copy of this sheet is enclosed.
  - ☐ Charge the amount of as filing fee.
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  - ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: **February 23, 1999**

Michael I. Stewart (24,973)
Signature

cc:

P01LARGE/REV06

TITLE OF INVENTION

EXPRESSING GP140 FRAGMENT OF PRIMARY HIV-1 ISOLATE

FIELD OF INVENTION

The present invention relates to the field of immunology, specifically HIV Vaccine Technology, and, in particular, is concerned with expressing the extracellular fragment of the envelope gene, gp140, of a primary human immunodeficiency virus type 1 (HIV-1) isolate.

BACKGROUND OF THE INVENTION

Acquired immunodeficiency syndrome (AIDS) is a disease which is the ultimate result of infection with human immunodeficiency virus (HIV). Currently, there is no effective vaccine which can protect the human population from HIV infection and hence the development of an efficacious HIV-vaccine and protocol for administering the same is urgently required. Previously, HIV-1 particles exhaustively inactivated by chemical treatments, a vaccinia vector encoding the whole envelope gene (gp140) of HIV-1, and purified recombinant gp120 have been evaluated as candidate HIV vaccines. Although inactivated HIV-1 virus preparations elicited a T-cell-mediated Delayed-Type Hypersensitivity (DTH) reaction in humans, and vaccinia/gp160 and gp120 recombinant vaccine candidates induced virus neutralizing antibodies, non of these immunogens have been shown to be efficacious human HIV vaccines (ref. 1, throughout this specification, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately following the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). The inventors' interest in HIV vaccinology is to develop immunogenic and cost-effective HIV-1 DNA vaccines and consider that

their use alone or in conjunction with other forms of HIV-1 vaccine candidates will lead to the elicitation of more effective immune responses against HIV-1.

There has previously been described in granted European Patent No. 470,980 and U.S. Patent No. 5,639,854, assigned to the assignee hereof, the disclosures of which are incorporated herein by reference, *inter alia*, the identification and characterization of a T-cell epitope of the core protein, p24E, of HIV-1. There has further been described in granted U.S. Patents Nos. 5,759,769 and 5,795,955, assigned to the assignee hereof, and disclosures of which are incorporated by reference, the use of the T-cell epitope in the construction of immunogenic synthetic HIV-1 chimeric peptides comprising p24E linked to amino acid sequences of different B-cell epitopes of an envelope or core protein of HIV-1.

SUMMARY OF THE INVENTION

The present effort has turned to design and construct HIV DNA-based immunogens capable of eliciting cell-mediated immunity (CMI). In this context, the inventors have focused interest on the extracellular envelope fragment, gp140, expressed in a primary HIV-1 isolate, HIV-1 (BX08), for the reason that this protein is rich in motifs restricted to both the murine and human Major Histocompatibility Complex (MHC) class 1 alleles. Upon immunization with an appropriately constructed immunogen expressing the gp140 protein leads to the generation of peptides with class 1 binding capability to allow the induction of HIV-1-specific CTLs capable of killing virus infected cells to limit infection.

The invention described by the inventors is that they have found a plasmid designated, pCMV.gp140.BX08, expressing the gp140 gene under the control of a CMV promotor was immunogenic in BALB/c mice in the elicitation of CTL response directed against multiple epitopes of the gp140 protein that are restricted to different H-2^d class 1 gene products.

Accordingly, in one aspect of the present invention, there is provided a vector, comprising a gene encoding the extracellular fragments of gp140 of a primary HIV-1 isolate, preferably BX08, under the control of a promotor for

expression of the gene product in a host organism, thereby eliciting a cytotoxic T-cell response.

The promotor thereby is the cytomegalovirus promotor. The vector is preferably one having the identifying characteristics of plasmid pCMV.gp140.BX08, as shown in Figure 1.

The invention further includes an immunogenic composition containing the vector as well as a method of generating a cytotoxic T-cell response in a host by administering to the host the immunogenic composition provided herein. Such immunogenic composition may be formulated for intramuscular immunization with a suitable carrier or may be formulated for gene gun delivery with gold particles.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows details of the elements of plasmid pCMV.gp140.BX08;

Figure 2 shows the nucleotide (SEQ ID No: 1) and deduced amino acid sequence (SEQ ID No: 2) of the gp140 open reading frame of the plasmid pCMV.gp140.BX08;

Figures 3A, 3B and 3C show the effector responses elicited by intramuscular injection of the plasmid, pCMV.gp140.BX08, in BALB/c mice; and

Figures 4A, 4B and 4C show the effector responses elicited by gene gun delivery of the plasmid, pCMV.gp140.BX08, in BALB/c mice.

DETAILED DESCRIPTION OF THE INVENTION

A DNA immunogen is constructed using recombinant DNA technology to molecularly clone a gene of interest into a plasmid expression vector. A unique feature involving vaccination with a DNA-based immunogen is that, once delivered into a cell, the intracellular production of the immunogen favours the induction of MHC class 1-restricted cytotoxic T-cells as compared to other forms of vaccination involving the use of killed whole cell and formulated sub-unit immunogens, which tend to favour the elicitation of MHC class 2-restricted immuno-regulatory responses in the majority of cases studied (ref. 2). In this

context, it is, therefore, favourable to use DNA technology to construct naked DNA immunogens for vaccination purposes in order to optimize the induction of cellular effector response against intracellular organisms, such as viruses as well as certain tumours. The other advantages DNA vaccines offer include: (i) the ease to produce; and (ii) their stability over a wide temperature range.

A common model which has been used recently to predict murine and human CTL antigenic determinants has involved the identification of binding motifs for the respective MHC class 1 molecules from the primary sequences of the native protein molecules (see refs. 3 to 5). Thus, it has been proposed that motifs which are most favoured to bind and lodge into the peptide-binding groove of the H-2D^d gene product is usually 8 to 10 amino acids long. In the majority of cases, these peptides are found to contain anchor residues, such as glycine and proline (GP), at positions 2 and 3 near the amino- (N-) terminus, and either a leucine or phenylalanine at the carboxy- (C-) terminus, which serve to interact with the respective 'pockets' of the peptide-binding groove of a membrane-bound H-2D^d molecule. The motifs restricted to the other class 1 allele, K^d, of the H-2^d haplotype were reported to contain a tyrosine at position 2, and could be an isoleucine, valine or leucine at the C-terminus. Studies of the peptides isolated from the human MHC class 1 molecules, HLA-A0201, had similarly revealed that the anchor residues were leucine or methionine at position 2 and valine or leucine at the C-terminus in the majority of cases.

The suitability of the HIV-1(BX08) gp140 gene product as a CTL-inducing immunogen was assessed by prediction algorithms to determine the number of both the murine and human MHC class 1-restricted binding motifs it contained. The amino acid sequences of the binding motifs and the designation of the peptides representing them are shown in Table 1 below. The presence of binding motifs towards the different H-2^d restricted class 1 alleles, i.e. D^d and K^d, allows the immunogenicity of a plasmid, pCMV.gp140.BX08, expressing gp140 of HIV-1 of the primary isolate, BX08, and constructed as described in the Example below, to be studied in the inbred mouse strain BALB/c of the H-2^d hapotype. The elements and restriction sites of plasmid pCMV.gp140.BX08 are

shown in Figure 1. The nucleotide (SEQ ID No: 1) and the deduced amino acid sequences (SEQ ID No: 2) of the gp140 open reading frame of the plasmid pCMV.gp140.BX08 is shown in Figure 2, which appear to be unique sequences.

The location of several binding motifs against the human MHC class 1 allele, HLA-A0201, as seen in Table 1, implied that, under an appropriate immunization regimen, the plasmid has the potential to elicit CTL response directed to these epitopes in the context of this class 1 molecule in human subjects.

The immunogenicity of the plasmid pCMV.gp140.BX08 was studied in BALB/c mice. The results of the study involving three injections of the plasmid at 100.0 μg per dose using the intramuscular route are shown in Figure 3. Upon *in vitro* re-stimulation of the spleenocytes of the plasmid-immunized animals with irradiated autologous LPS blasts pulsed individually with the D^d- and K^d-restricted motif containing peptides, namely, CLP-501 and CLP-504 (SEQ ID Nos.: 3, 5), respectively, it was found that CTLs were generated that killed P815 targets presented with the respective peptides (Figs. 3A and 3B). The amino acid sequences of the peptides are shown in Table 1. A comparison of the magnitude of the responses at the same effector to target (E:T) ratio revealed that the D^d-restricted response to the CLP-501 peptide is immuno-dominant and that the K^d-restricted response to the CLP-504 peptide is sub-dominant. The *in vitro* re-stimulation leading to the expansion of the effectors was specific because the addition of the same number of irradiated LPS blasts alone (not treated with peptide) did not lead to any generation of effectors in the bulk culture able to kill either of the specific targets tested. The findings that the control group of mice injected with the pCMV vector without the gp140 insert alone failed to generate any of the two sub-populations of CTLs (Fig. 3C) confirmed that the plasmid, pCMV.gp140.BX08, was indeed immunogenic.

The pCMV.gp140.BX08 plasmid, when delivered with the gene gun, was similarly found to be immunogenic. The results shown in Figure 4 show that following two injections at a dose of 0.7 μg of the plasmid, and using the same *in vitro* re-stimulation condition described above that CTLs recognizing the CLP-501

and CLP-504 peptides were detected (Figures 4A and 4B), while no effector response was elicited by the group of animals given the vector, pCMV, alone (Figure 4C).

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of HIV infections. A further non-limiting discussion of such uses is further presented below.

Immunogenic compositions, including vaccines, containing the DNA vector may be prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The DNA vector may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid liposome (for example, as described in WO 93/24640) or the DNA vector may be associated with an adjuvant, as described in more detail below.

Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435 describes compositions for genetic immunization comprising cationic lipids and polynucleotides.

Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be used with the vector.

Polynucleotide immunogenic preparation may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 describes a particular carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactideco-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co-glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake of vaccine across the nasal mucosa. The delivery vehicle may additionally contain and absorption enhancer.

In particular embodiments of the present invention, the vector may be delivered in conjunction with a targeting molecule to target the vector to selected cells including cells of the immune system.

The vectors may be delivered to the host by a variety of procedures, for example, Tang et al (ref. 6) discloses that introduction of gold microprojectiles coated with DNA encoding bovine growth hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth et al. (ref. 7) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

Biological Deposits

Certain vectors that contain nucleic acid coding for a high molecular weight protein of a non-typeable strain of *Haemophilus* that are described and referred to herein have been deposited with the America Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA, pursuant the Budapest Treaty and prior to the filing of this application. Samples of the deposited vectors will become available to the public and all restrictions imposed or access to the deposits will be received upon grant of a patent based on this United States patent application. In addition, the deposit will be replaced if viable samples cannot be dispensed by the Depository. The invention described and claimed herein is not limited in scope by the biological

materials deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or similar vectors that contain nucleic acid which encodes equivalent or similar antigens as described in this application are within the scope of the invention.

Deposit Summary

Plasmid	ATCC	Deposited Date
pCMV.gp140.BX08		

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

Methods of plasmid construction, peptide synthesis, cell culture, CTL assay and other testing procedures that are not explicitly described in this disclosure are amply reported in the scientific literature and are will within the scope of those skilled in the art.

Example 1

This Example illustrates the construction of the plasmid, pCMV.gp140.BX08.

The plasmid, pCMV.gp140.BX08, contains sequence segments from various sources, and the elements of construction are depicted in Figure 1. The nucleotide (SEQ ID No: 1) and derived amino acid sequences (SEQ ID No: 2) of the gp140 open reading frame of the plasmid are shown in Figure 2.

The prokaryotic vector pBluescript SK (Stratagene) is the backbone of the plasmid pCMV.gp140.BX08 and was modified by the replacement of the Amp^R

with Kan^R gene and the deletion of the f1 and the LacZ region. To achieve the desired modifications, the sequence between AhdI (nucleotide 2,041) and SacI (nucleotide 759) of pBluescript SK, which contains the Amp^R, f1 origin and the LacZ, was deleted. A 1.2 kb PstI fragment from the plasmid pUC-4K (Pharmacia) containing the Kan^R gene, was blunt end ligated to the AhdI site of pBluescript SK in a counter-clockwise orientation relative to it's transcription. A 1.6 kb SspI/PstI DNA fragment containing the human cytomegalovirus immediate-early gene promotor, enhancer and intron A sequences (CMV) was ligated to the other end of the Kan^R gene so that the transcription from the CMV promotor proceeds in the clockwise orientation. A synthetic oligonucleotide segment containing translation initiation sequence and sequences encoding the human tissue plasminogen activator signal peptide (TPA) was used to link the CMV promotor and the sequences encoding the gp140 of the primary isolate HIV-1_{BX08}. The gp140 sequence encodes a portion of the envelope protein between amino acid 33 and 666 which ends before the transmembrane domain of gp41 (see Figure 2). A translation termination codon was placed at the end of the gp140 coding sequence. Next to the gp140 coding region is a 0.2 kb fragment containing the bovine growth hormone (BGH) polyadenylation signal sequence that is PCR amplified from pRC/CMV (Invitrogen). A remnant 80 bp DNA segment from the SV40 polyadenylation signal remained between the BGH poly A sequence and the SacI site of pBluescript SK due to DNA manipulation and it serves no purpose in this plasmid.

The pCMV.gp140.BX08 construct was introduced into HB101 competent cells according to manufacturer's recommendations (GibcoBRL). Correct molecular clones were identified by restriction and sequencing analysis and their expression of gp140 was examined in transient transfections followed by Western blot analysis.

All DNAs used for immunizations were prepared using EndoFree Plasmid Kit (Qiagen). For intramuscular immunizations in mice, 100 µg of pCMV.gp140.BX08, in 100 µl PBS was injected into the tibialis anterior muscles at 4 weeks intervals. Gene gun immunizations were accomplished with the Helios

Gene Gun System (Biorad). Cartridges were prepared according to manufacturer's recommendations. Specifically, each cartridge was made to contain 0.7 μg of the DNA and 0.5 mg gold. Immunizations were carried out by applying two cartridges to each animal onto the shaved abdominal area at 4 week intervals.

Example 2

This Example illustrates the synthesis of peptides.

Solid phase peptide synthesis of peptide CLP-501 and CLP-504 were conducted on an ABI 430A automated peptide synthesizer according to the manufacturer's standard protocols. The peptides were cleaved from solid support by treatment with liquid hydrogen fluoride in the presence of thiocresole, anisole, and methyl sulfide. The crude products were extracted with trifluoroacetic acid (TFA) and precipitated with diethyl ether.

The amino acid sequences of these peptides are shown in Table 1.

Example 3

This Example illustrates *in vitro* cell culture protocols to re-stimulate and expand CTLs and assay for their effector functions.

Spleens of BALB/c mice injected with the plasmid, pCMV.gp140.BX08, prepared and formulated as described in Example 1, using the intramuscular route or gene gun delivery method, were removed 10 to 11 days post final booster injection. Spleenocytes at 3.0×10^7 were co-cultured with 1.3×10^7 autogolous LPS blasts which had been pulsed with the test peptide for 5hr at 37°C and irradiated at 3000 rads in 10.0 ml of complete medium (RPMI 1640 supplemented with 10.0% 56°C heat-inactivated bovine serum, 120.0 units per ml of penicillin G sodium, 120.0 μg per ml of streptomycin sulphate and 0.35 mg per ml of L-glutamine) in a 25 cm^2 tissue culture flask. The cultures were kept at 37°C in a humidified CO_2 incubator for days, and the responders were then tested against peptide-pulsed P815 target cells in a standard *in vitro* 4 hr CTL assay as follows:

The responders were harvested from the 7-day cultures and washed once with RPMI 1640 medium without added bovine serum. The positive target was created by incubating 3 to 5 x 10^6 P815 cells with 100.0 μg of the specified peptide overnight in a 26°C water bath. The target cells were then labeled with ^{51}Cr at 250.0 uCi per 1 x 10^6 cells in the presence of 25.0 μg of the same test peptides for 60 to 75 minutes at 26°C. After washing twice with complete medium to remove excess ^{51}Cr, the targets were incubated at 2.5 x 10^3 with different numbers of the responders per well in a V-bottomed 96 will tissue culture plates for 4 hr in a 37°C $C0_2$ incubator. Half amount of the supernatant from each micro-assay culture was then removed and counted for radioactivity. Results were expressed as % which was calculated using the equation:

% lysis = (spontaneous lysis in cpm of experimental sample – spontaneous lysis in cpm of labeled target cells alone) devided by (totally lysis in com of target cells alone – spontaneous lysis in cpm of target cells alone) x 100.

The results obtained employing intramuscular injection are shown in Figures 3A, 3B and 3C while those obtained employing the gene gun delivery are shown in Figures 4A, 4B and 4C and are disable alone.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a novel plasmid expressing, *in vitro* and *in vivo*, the gp140 protein of the primary HIV-1 isolate BX08 and the generation of MHC class 1-restricted cytotoxic T-cells in animals. Modifications are possible within the scope of this invention.

Table 1

MHC class 1-restricted motifs of the extracellular envelope fragment, gp140, of HIV-1(BX08)

H-2^d-restricted *				HLA-A0201-restricted **
Peptide ***	D^d-	Peptide ***	K^d-	
CLP-501	IGPGRAFYTT (274-283) (SEQ ID No:3)	CLP-503	AYDTEVHNV (29-37) (SEQ ID No:4)	KLTPLCVTL (91-98) (SEQ ID No:10)
		CLP-504	FYSLKIVPI (141-149) (SEQ ID No:5)	TLFRVAIKL (305-313) (SEQ ID No:11)
		CLP-505	LYKYKVVKI (443-451) (SEQ ID No:6)	TLTVQARQL (403-411) (SEQ ID No:12)
		CLP-506	KYKVVKIEPL (445-454) (SEQ ID No:7)	TLTVQARAL (496-504) (SEQ ID No:13)
		CLP-507	RYLQDQRFL (545-553) (SEQ ID No:8)	QLQARVLAL (535-543) (SEQ ID No:14)
		CLP-508	NYTEIIYSL (597-605) (SEQ ID No:9)	

* or ** Anchors residues were typed in bolded letters.

*** Peptides chosen for the study reported herein are bolded.

REFERENCES

1. B.J. Spalding. Biotechnology, vol. 10, pp 24-28, 1992
2. H.L. Robinson and C.A.T. Torres. Seminars in Immunology, vol. 9, pp 271-283, 1997.
3. Ian A. Wilson and Daved H. Fremont. Seminars in Immunology, vol. 5, pp 75-80, 1993.
4. Kristen Falk and Olaf Rotzschke. Seminars in Immunology. Vol. 5, pp 81-94, 1993.
5. Victor H. Englefard. Current Opinion in Immunology, Vol. 6, pp 13-23, 1994.
6. Tang et al, Nature, 1992, 356:152-154.
7. Furth et al, Analytical Biochemistry, 1992, 205:365-368.

CLAIMS

What we claim is:

1. A vector, comprising a gene encoding the extracellular fragment of gp140 of a primary HIV-1 isolate under the control of a promotor for expression of the gene product in a host organism.

2. The vector of claim 1, wherein said primary HIV-1 isolate is BX08.

3. The vector of claim 2, wherein the promoter is the cytomegalovirus promotor.

4. The vector of claim 1, which has the identifying characteristics of pCMV.gp140.BX08 (ATCC No. ___), as shown in Figure 1.

5. An immunogenic composition comprising a vector comprising a gene encoding the extracellular fragment of gp140 of a primary HIV-1 isolate under the control of a promotor for expression of the gene product in a host organism.

6. The immunogenic compositions of claim 5, wherein said primary HIV-1 isolate is BX08.

7. The immunogenic compositions of claim 5, wherein the promoter is the cytomegalovirus promotor.

8. The immunogenic composition of claim 5, wherein the vector is pCMV.gp140.BX08.

9. The immunogenic composition of claim 5 formulated for intramuscular immunization with a pharmaceutically-acceptable liquid carrier.

10. The immunogenic composition of claim 4 formulated for gene gun delivery with gold particles

11. A method of generating a cytotoxic T-cell response in a host, which comprises administering to the host the immunogenic composition of claim 5.

12. A peptide having an amino acid sequence selected from the group consisting of SEQ ID Nos.:3 to 14, as shown in Table 1.

13. The peptide of claim 12, having SEQ ID No.:3.

14. The peptide of claim 12, having SEQ ID No.:5.

ABSTRACT OF THE DISCLOSURE

A vector for eliciting an immune response to a host comprising a gene encoding the gp140 protein of the primary isolate of HIV-1, BX08, under the control of a promotor for expression of the protein in the host, specifically plasmid pCMV.gp140.BX08. Murine and human MHC class 1-restricted binding motifs contained in BX08 are identified.

Docket No.
1038-920 MIS:jb

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

EXPRESSING GP140 FRAGMENT OF PRIMARY HIV-1 ISOLATE

the specification of which

(check one)

☒ is attached hereto.

☐ was filed on _____ as United States Application No. or PCT International Application Number _____

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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Fifth inventor's signature — Date

Residence

Citizenship

Post Office Address

Full name of sixth inventor, if any

Sixth inventor's signature — Date

Residence

Citizenship

Post Office Address

Figure 1. The pCMV.gp140.BX08 plasmid

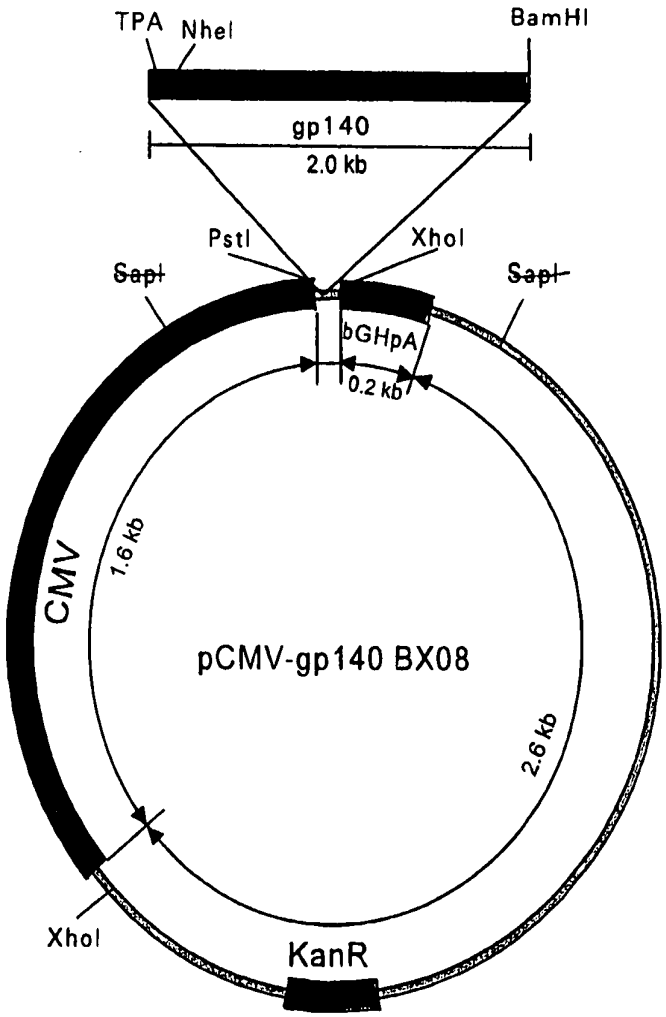

Figure 2. Nucleotide sequence used for the construction of the pCMV.gp140. BX08 plasmid

```
 +1 M  D  A  M  K  R  G  L  C  C  V  L  L  L  C  G
  1 ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG CTG CTG CTG TGT GGA
    TAC CTA CGT TAC TTC TCT CCC GAG ACG ACA CAC GAC GAC GAC ACA CCT

 +1 A  V  F  V  S  A  S  L  W  V  T  V  Y  Y  G  V
 49 GCA GTC TTC GTT TCG GCT AGC TTG TGG GTC ACA GTC TAT TAT GGG GTA
    CGT CAG AAG CAA AGC CGA TCG AAC ACC CAG TGT CAG ATA ATA CCC CAT

 +1 P  V  W  K  E  A  T  T  T  L  F  C  A  S  D  A
 97 CCT GTG TGG AAA GAA GCA ACC ACC ACT CTA TTT TGT GCA TCA GAT GCT
    GGA CAC ACC TTT CTT CGT TGG TGG TGA GAT AAA ACA CGT AGT CTA CGA

 +1 K  A  Y  D  T  E  V  H  N  V  W  A  T  H  A  C
145 AAA GCA TAT GAT ACA GAA GTA CAT AAT GTT TGG GCC ACA CAT GCC TGT
    TTT CGT ATA CTA TGT CTT CAT GTA TTA CAA ACC CGG TGT GTA CGG ACA

 +1 V  P  T  D  P  N  P  Q  E  V  V  L  G  N  V  T
193 GTA CCC ACA GAC CCC AAC CCA CAA GAA GTA GTA TTG GGA AAT GTG ACA
    CAT GGG TGT CTG GGG TTG GGT GTT CTT CAT CAT AAC CCT TTA CAC TGT

 +1 E  N  F  N  M  W  K  N  N  M  V  E  Q  M  H  E
241 GAA AAT TTT AAC ATG GGG AAA AAT AAC ATG GTA GAA CAG ATG CAT GAA
    CTT TTA AAA TTG TAC CCC TTT TTA TTG TAC CAT CTT GTC TAC GTA CTT

 +1 D  I  I  S  L  W  D  Q  S  L  K  P  C  V  K  L
289 GAT ATA ATT AGT TTA TGG GAT CAA AGC CTA AAG CCA TGT GTA AAA TTA
    CTA TAT TAA TCA AAT ACC CTA GTT TCG GAT TTC GGT ACA CAT TTT AAT

 +1 T  P  L  C  V  T  L  N  C  T  K  L  K  N  S  T
337 ACC CCA CTC TGT GTT ACT TTA AAT TGC ACT AAG TTG AAG AAT AGT ACT
    TGG GGT GAG ACA CAA TGA AAT TTA ACG TGA TTC AAC TTC TTA TCA TGA

 +1 D  T  M  N  T  R  W  G  T  Q  E  M  K  N  C  S
385 GAT ACC AAT AAT ACT AGA TGG GGA ACA CAA GAA ATG AAA AAC TGC TCT
    CTA TGG TTA TTA TGA TCT ACC CCT TGT GTT CTT TAC TTT TTG ACG AGA

 +1 F  N  I  S  T  S  V  R  N  K  M  K  R  E  Y  A
433 TTC AAC ATC AGC ACA AGT GTA AGA AAT AAG ATG AAG AGA GAA TAT GCA
    AAG TTG TAG TCG TGT TCA CAT TCT TTA TTC TAC TTC TCT CTT ATA CGT

 +1 L  F  Y  S  L  D  I  V  P  I  D  N  D  N  T  S
481 CTT TTT TAT AGT CTT GAT ATA GTA CCA ATA GAT AAT GAT AAT ACT AGC
    GAA AAA ATA TCA GAA CTA TAT CAT GGT TAT CTA TTA CTA TTA TGA TCG

 +1 Y  R  L  R  S  C  N  T  S  I  I  T  Q  A  C  P
529 TAT AGG TTA AGA AGT TGT AAT ACC TCA ATC ATT ACA CAG GCC TGT CCA
    ATA TCC AAT TCT TCA ACA TTA TGG AGT TAG TAA TGT GTC CGG ACA GGT

 +1 K  V  S  F  E  P  I  P  I  H  F  C  A  P  A  G
577 AAG GTA TCC TTT GAG CCA ATT CCC ATA CAT TTT TGT GCC CGG GCT GGT
    TTC CAT AGG AAA CTC GGT TAA GGG TAT GTA AAA ACA CGG GGC CGA CCA

 +1 F  A  I  L  K  C  N  N  K  T  F  N  G  T  G  P
625 TTT GCG ATT CTA AAG TGT AAC AAT AAA ACG TTC AAT GGA ACA GGA CCA
    AAA CGC TAA GAT TTC ACA TTG TTA TTT TGC AAG TTA CCT TGT CCT GGT

 +1 C  T  N  V  S  T  V  Q  C  T  H  G  I  R  P  V
673 TGT ACA AAT GTC AGC ACA GTA CAA TGT ACA CAT GGA ATT AGG CCA GTA
    ACA TGT TTA CAG TCG TGT CAT GTT ACA TGT GTA CCT TAA TCC GGT CAT

 +1 V  S  T  Q  L  L  L  N  G  S  L  A  E  E  E  V
721 GTA TCA ACT CAA CTG CTG TTA AAT GGC AGC CTA GCA GAA GAA GAG GTA
    CAT AGT TGA GTT GAC GAC AAT TTA CCG TCG GAT CGT CTT CTT CTC CAT

 +1 V  I  R  S  E  N  F  T  N  N  A  K  T  I  I  V
769 GTA ATT AGA TCT GAA AAT TTC ACA AAC AAT GCT AAA ACC ATA ATA GTA
    CAT TAA TCT AGA CTT TTA AAG TGT TTG TTA CGA TTT TGG TAT TAT CAT

 +1 Q  L  N  E  S  V  E  I  N  C  T  R  P  N  N  N
817 CAG CTA AAT GAA TCT GTA GAA ATT AAT TGT ACA AGA CCC AAC AAC AAT
    GTC GAT TTA CTT AGA CAT CTT TAA TTA ACA TGT TCT GGG TTG TTG TTA

 +1 T  R  K  S  I  H  I  G  P  G  R  A  F  Y  T  T
865 ACA AGA AAA AGT ATA CAT ATA GGA CCA GGG AGA GCA TTT TAT ACA ACA
    TGT TCT TTT TCA TAT GTA TAT CCT GGT CCC TCT CGT AAA ATA TGT TGT

 +1 G  D  I  I  G  D  I  R  Q  A  H  C  N  I  S  R
913 GGA GAT ATA ATA GGA GAT ATA AGA CAA GCA CAT TGT AAC ATT AGT AGA
    CCT CTA TAT TAT CCT CTA TAT TCT GTT CGT GTA ACA TTG TAA TCA TCT

 +1 T  N  W  T  N  T  L  K  R  V  A  E  K  L  R  E
961 ACA AAC TGG ACT AAC ACT TTA AAA AGG GTA GCT GAA AAA TTA AGA GAA
    TGT TTG ACC TGA TTG TGA AAT TTT TCC CAT CGA CTT TTT AAT TCT CTT

 +1 K  F  N  N  T  I  V  F  N  Q  S  S  G  G  D
1009 AAA TTT AAT AAT ACA ACA ATA GTC TTT AAT CAA TCC TCA GGA GGG GAC
     TTT AAA TTA TTA TGT TGT TAT CAG AAA TTA GTT AGG AGT CCT CCC CTG

 +1 P  E  I  V  M  H  S  F  N  C  G  G  E  F  F  Y
1057 CCA GAA ATT GTA ATG CAC AGT TTT AAT TGT GGA GGG GAA TTT TTC TAC
     GGT CTT TAA CAT TAC GTG TCA AAA TTA ACA CCT CCC CTT AAA AAG ATG

 +1 C  N  T  T  Q  L  F  N  S  T  W  N  E  T  N  S
1105 TGT AAT ACA ACA CAA CTG TTT AAT AGT ACT TGG AAT GAA ACT AAC AGT
     ACA TTA TGT TGT GTT GAC AAA TTA TCA TGA ACC TTA CTT TGA TTG TCA

 +1 E  G  N  I  T  S  G  T  I  T  L  P  C  R  I  K
1153 GAA GGA AAT ATC ACC AGT GGA ACT ATA ACA CTC CCA TGC AGA ATA AAA
     CTT CCT TTA TAG TGG TCA CCT TGA TAT TGT GAG GGT ACG TCT TAT TTT

 +1 Q  I  I  N  M  W  Q  E  V  G  K  A  M  Y  A  P
1201 CAA ATT ATA AAC ATG TGG CAG GAA GTA GGA AAA GCA ATG TAT GCC CCT
     GTT TAA TAT TTG TAC ACC GTC CTT CAT CCT TTT CGT TAC ATA CGG GGA

 +1 P  I  G  G  Q  I  K  C  L  S  N  I  T  G  L  L
1249 CCC ATC GGA GGA CAA ATT AAA TGT TTG TCA AAC ATC ACA GGG CTG TTA
     GGG TAG CCT CCT GTT TAA TTT ACA AAC AGT TTG TAG TGT CCC GAC AAT

 +1 L  T  R  D  G  G  S  D  N  S  S  S  G  K  E  I
1297 TTA ACA AGA GAT GGT GGT AGT GAT AAC AGT AGT AGT GGG AAA GAG ATC
     AAT TGT TCT CTA CCA CCA TCA CTA TTG TCA TCA TCA CCC TTT CTC TAG

 +1 F  R  P  G  G  G  D  M  R  D  N  W  R  S  E  L
1345 TTC AGA CCT GGA GGG GGA GAT ATG AGG GAC AAT TGG AGA AGT GAA TTA
     AAG TCT GGA CCT CCC CCT CTA TAC TCC CTG TTA ACC TCT TCA CTT AAT

 +1 Y  K  Y  K  V  V  K  I  E  P  L  G  I  A  P  T
1393 TAT AAA TAT AAG GTA GTA AAA ATT GAA CCA TTA GGA ATA GCA CCC ACC
     ATA TTT ATA TTC CAT CAT TTT TAA CTT GGT AAT CCT TAT CGT GGG TGG

 +1 K  A  K  R  R  V  V  Q  R  E  K  R  A  V  G  I
1441 AAG GCA AAG AGA AGA GTG GTG CAG AGA GAA AAA AGA GCA GTG GGA ATA
     TTC CGT TTC TCT TCT CAC CAC GTC TCT CTT TTT TCT CGT CAC CCT TAT

 +1 G  A  M  F  L  G  F  L  G  A  A  G  S  T  M  G
1489 GGA GCC ATG TTC CTT GGG TTC TTG GGA GCA GCA GGA AGC ACT ATG GGC
     CCT CGG TAC AAG GAA CCC AAG AAC CCT CGT CGT CCT TCG TGA TAC CCG

 +1 A  A  S  L  T  L  T  V  Q  A  R  Q  L  L  S  G
1537 GCA GCG TCA CTA ACG CTG ACG GTA CAG GCC AGA CAA TTA TTG TCT GGT
     CGT CGC AGT GAT TGC GAC TGC CAT GTC CGG TCT GTT AAT AAC AGA CCA

 +1 I  V  Q  Q  Q  N  N  L  L  R  A  I  E  A  Q  Q
1585 ATA GTG CAG CAG CAA AAC AAT TTG CTG AGG GCT ATT GAG GCG CAA CAG
     TAT CAC GTC GTC GTT TTG TTA AAC GAC TCC CGA TAA CTC CGC GTT GTC

 +1 H  L  L  Q  L  T  V  W  G  I  K  Q  L  Q  A  R
1633 CAC CTG TTG CAA CTC ACA GTC TGG GGC ATC AAG CAG CTC CAG GCA AGA
     GTG GAC AAC GTT GAG TGT CAG ACC CCG TAG TTC GTC GAG GTC CGT TCT

 +1 V  L  A  L  E  R  Y  L  Q  D  Q  R  F  L  G  M
1681 GTC CTG GCT CTG GAA AGA TAC CTA CAG GAT CAA CGG TTC CTA GGG ATG
     CAG GAC CGA GAC CTT TCT ATG GAT GTC CTA GTT GCC AAG GAT CCC TAC

 +1 W  G  C  S  G  K  L  I  C  T  T  A  V  P  W  N
1729 TGG GGT TGC TCT GGA AAA CTC ATC TGC ACC ACT GCT GTG CCT TGG AAT
     ACC CCA ACG AGA CCT TTT GAG TAG ACG TGG TGA CGA CAC GGA ACC TTA

 +1 A  S  W  S  N  K  N  L  S  Q  I  W  D  N  M  T
1777 GCT AGT TGG AGT AAT AAA AAT CTA AGT CAG ATT TGG GAT AAC ATG ACC
     CGA TCA ACC TCA TTA TTT TTA GAT TCA GTC TAA ACC CTA TTG TAC TGG

 +1 W  M  E  W  E  R  E  I  S  N  Y  T  E  I  I  Y
1825 TGG ATG GAG TGG GAG AGA GAA ATA AGC AAT TAC ACA GAG ATA ATA TAT
     ACC TAC CTC ACC CTC TCT CTT TAT TCG TTA ATG TGT CTC TAT TAT ATA

 +1 S  L  I  E  E  S  Q  N  Q  Q  E  K  N  E  L  D
1873 AGC TTA ATT GAA GAA TCG CAG AAC CAA CAA GAA AAG AAT GAA CTA GAC
     TCG AAT TAA CTT CTT AGC GTC TTG GTT GTT CTT TTC TTA CTT GAT CTG

 +1 L  L  Q  L  D  K  W  A  S  L  W  N  W  F  D  I
1921 TTA TTA CAA TTG GAT AAG TGG GCA AGT TTG TGG AAT TGG TTT GAC ATA
     AAT AAT GTT AAC CTA TTC ACC CGT TCA AAC ACC TTA ACC AAA CTG TAT

 +1 T
1969 ACA
     TGT
```

Note: shown is the translation of the sense strain with it's anti-sense strain below it that was cloned into the pCMV3 vector described in the text

Figure 3. Effector responses elicited by intramuscular injection of the plasmid, pCMV.gp140.BX08, into BALB/c mice

	Figure 3A	Figure 3B	Figure 3C
Immunization:	100.0 ug pCMV.gp140.BX08 + 100.0 ug pCMV	100.0 ug pCMV.gp140.BX08 100.0 ug pCMV	100.0 ug pCMV
***In vitro* re-stimulation:**	with autologous CLP-501 pulsed LPS blasts (■)	with autologous CLP-504 pulsed LPS blasts(●)	with autologous CLP-501 pulsed LPS blasts (▲)
	with autologous LPS blasts (▲)		with autologous LPS-504 pulsed LPS blasts (●)
Target:	CLP-501-pulsed P815	CLP-504-pulsed P815	CLP-501-pulsed P815 for CLP-501-pulsed blast re-stimulation CLP-504-pulsed P815 for CLP-504-pulsed blast re-stimulation

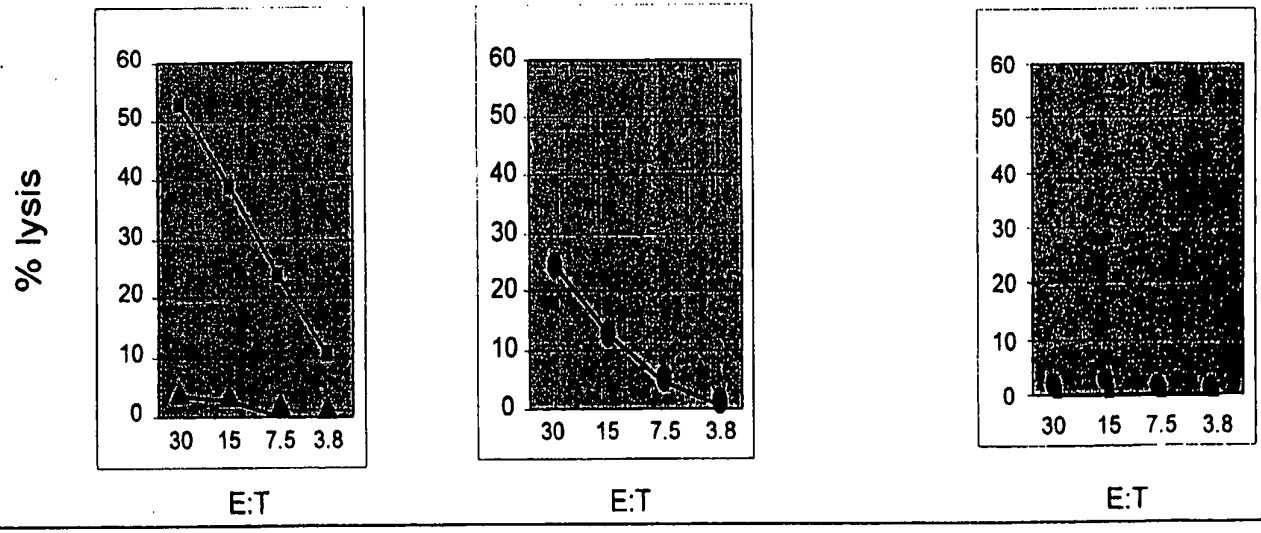

Figure 4. Effector responses elicited by gene gun delivery of the plasmid, pCMV.gp140.BX08, into BALB/c mice

	Figure 4A	Figure 4B	Figure 4C
Immunization:	0.7 ug pCMV.gp140.BX08 + 0.7 pCMV	0.7 ug pCMV.gp140.BX08 + 0.7 ug pCMV	1.4 ug pCMV
***In vitro* re-stimulation:**	with autologous CLP-501 pulsed LPS blasts (■) with autologous LPS blasts (▲)	with autologous CLP-504 pulsed LPS blasts (●)	with autologous CLP-501 pulsed LPS blasts (■) with autologous LPS-504 pulsed LPS blasts ( )
Target:	CLP-501-pulsed P815	CLP-504-pulsed P815	CLP-501-pulsed P815 for CLP-501-pulsed blast re-stimulation CLP-504-pulsed P815 for CLP-504-pulsed blast re-stimulation